

## **Liquid biopsy from Bile - a potential novel biomarker in biliary stenosis**

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### **Background & objectives**

To establish the diagnosis of malignant bile duct obstructions is frequently challenging in clinical routine. Endoscopic retrograde cholangiography (ERC) including brush cytology and forceps biopsy remains the diagnostic gold standard, but sensitivity of combined brush cytology and forceps biopsy commonly does not exceed 60-70%. However, bile can harbor DNA fragments being released by tumor cells and, thus, tumor specific mutations in DNA fragments from bile might help the classification and improve diagnostic yield for malignant biliary stenosis.

The aim of this study was to evaluate if liquid biopsy-based digital droplet PCR (ddPCR) facilitates detection of common oncogenic mutations from bile and plasma in malignant biliary stenosis and improves sensitivity of conventional cytology and histology.

### **Methods**

The most frequent mutations in bile duct malignancy were identified in the COSMIC database. We established singleplex ddPCR assays for KRAS G12R/G12V/G13D/Q61L. Furthermore, novel ddPCR assays are being established. Collection of bile samples and clinical data is ongoing. Bile samples as well as plasma will be measured in 100 patients with unknown bile duct stenosis. The mutations measured by ddPCR will be compared with clinical disease parameters, including cytology and histology results. In a pilot phase, we analyzed three bile samples from patients with bile duct stenosis.

### **Results**

In the pilot phase, singleplex ddPCR assays for KRAS G12R/G12V/G13D and Q61L were successfully validated. The KRAS G12V assay was subsequently analyzed in bile samples from three patients with malignant biliary stenosis. Two samples were positive for KRAS G12V, in one of the patients a G12V mutation was already known from tumor tissue. Interestingly, the other patient was obtained from a hepatic bifurcation stenosis without

previous diagnosis of a malignant tumor with missing histology results. The third sample measured correctly negative with matched tumor tissue.

## **Conclusion**

We detected tumor specific KRAS mutations by liquid biopsy of bile in patients with suspicious bile duct stenoses. Therefore, liquid biopsy from bile might be a promising approach to improve diagnosis of malignancies in these patients.